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Necrosis: Linking the Inflammasome to Inflammation

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In this issue of *Cell Reports*, Cullen et al. demonstrate that the release of mature interleukin-1β relies on necrotic plasma membrane permeabilization. Thus, caspases may have evolved to modulate the inflammatory potential of cell death, not to execute it.

Interleukin-1β (IL-1β) and IL-18 are crucial to the inflammatory response, whether this is elicited by microbial stimuli or endogenous danger signals (Kroemer et al., 2013). Secretion of biologically active IL-1β and IL-18 proceeds via a two-step mechanism. First, pro-IL-1β and pro-IL-18 are synthesized upon the Toll-like receptor 4 (TLR4)-driven transactivation of *IL1B* and *IL18* ("signal I"). Second, a multiprotein complex commonly known as the "NLRP3 inflammasome" catalyzes the proteolytic maturation of IL-1β and IL-18, hence promoting their release ("signal II") (Latz et al., 2013). Several stimuli, including ATP, ouabain (a plasma membrane Na^+/K^+-ATPase inhibitor), various immunological adjuvants (e.g., *L*-leucyl-*L*-leucine methyl ester [LLOMe]), and pore-forming toxins (e.g., streptolysin O [SLO], nigericin, valinomycin), activate the NLRP3 inflammasome. The release of biologically active IL-1β and IL-18 in response to these agents has been linked to K^+ ion efflux from the cytosol. Accordingly, the artificial increase of extracellular K^+ levels inhibits IL-1β and IL-18 processing (Latz et al., 2013). Until now, however, a unified model explaining how mature IL-1β and IL-18 are secreted was missing. In this issue of *Cell Reports*, Cullen et al. demonstrate that the maturation and release of IL-1β critically impinge on necrotic plasma membrane permeabilization (PMP) (Cullen et al., 2015).

Cullen et al. test the simple hypothesis that agents promoting IL-1β secretion do so by triggering necrosis, which is associated with rapid PMP (Galluzzi et al., 2014a). Indeed, SLO, nigericin, valinomycin, ATP, ouabain, and LLOMe all cause PMP in THP-1 human monocytic cells, correlating with the release of active IL-1β upon priming with the TLR4 agonist lipopolysaccharide (LPS). The PMP-inducing activity of these molecules does not depend on LPS priming and is not blocked by pharmacological or genetic inhibition of the NLRP3 inflammasome. Conversely, the wide-spectrum caspase inhibitor Z-VAD-fmk, as well as knockdown of various NLRP3-inflammasome components, efficiently blocks IL-1β maturation and release from THP-1 cells primed with LPS and exposed to nigericin. These findings indicate that the cytotoxicity of SLO, nigericin, valinomycin, ATP, ouabain, and LLOMe does not rely on the activation of the NLRP3 inflammasome (and hence on IL-1β signaling). IL-1β release from some cell types, such as bone-marrow-derived macrophages (BMDMs), does not require signal II, meaning that BMDMs produce IL-1β directly upon LPS exposure. As hypothesized by Cullen et al., LPS causes rapid PMP in BDMCs, and the degree of PMP correlates with the amount of bioactive IL-1β detected in culture supernatants. Thus, when signal II is not required for IL-1β secretion, signal I alone promotes PMP.

The cytotoxic response of THP-1 cells to agents associated with signal II does not manifest morphological features of apoptosis and is independent of caspase 3 (CASP3) and CASP7 activation. Accordingly, CASP3 and CASP7 knockdown fails to exert cytoprotective effects in this setting. Similarly, neither necrostatin-1 (a pharmacological inhibitor of necroptosis) administration nor down-regulation of various components of the necroptotic machinery efficiently prevents PMP induction by nigericin or ouabain. On the basis of these observations, the authors conclude that signal II is associated with "conventional necrosis." However, the involvement of other forms of regulated necrosis such as mitochondrial permeability transition (MPT)-driven necrosis or parthanatos, which impinges on poly (ADP-ribose) polymerase 1 (PARP1) signaling (Galluzzi et al., 2014b), was not tested. The precise mechanisms whereby SLO, nigericin, valinomycin, ATP, ouabain, and LLOMe trigger necrosis remain unknown.

Irrespective of this unresolved issue, Cullen et al. demonstrate that the release of IL-1β from cells exposed to agents associated with signal II is non-specific; i.e., it is accompanied by the release of many other cytosolic proteins. Moreover, the authors elegantly rule out the possibilities that a subpopulation of necrosis-resistant cells secretes IL-1β and that pro-IL-1β is converted into biologically

active IL-1β extracellularly. Finally, they show that known inhibitors of IL-1β maturation and release, including excess extracellular K^+ ions and the antidiabetic drug glyburide, actually inhibit necrotic PMP. In summary, the observations by Cullen et al. suggest that necrosis is necessary and sufficient for the secretion of bioactive IL-1β as necrotic PMP allows for the efflux of K^+ ions required for NLRP3 activation, as well as for the unspecific release of IL-1β.

The work by Cullen et al. adds to growing evidence suggesting that caspases have not evolved as executioners of cell death (Galluzzi et al., 2015), but as a means to exacerbate (CASP1) or inhibit (CASP8, CASP9, CASP3) its inflammatory potential (Figure 1). Indeed, necroptosis (which has a robust inflammatory outcome) is suppressed by a multiprotein complex containing CASP8 (Pasparakis and Vandenabeele, 2015). CASP9-mediated CASP3 activation is required for dying cells to expose phosphatidylserine on the cell surface, resulting in rapid uptake by phagocytes (Segawa et al., 2014). Moreover, CASP3 actively inhibits pro-inflammatory type I interferon signaling elicited by MAVS in response to cytosolic mtDNA (Rongvaux et al., 2014; White et al., 2014). It will be interesting to investigate the fate of pro-IL-1β in LPS-primed monocytes driven to CASP3- or CASP8-dependent cell death, which is associated with delayed PMP. It is tempting to speculate (but remains to be formally demonstrated) that these cells will not release biologically active IL-1β, perhaps as a direct consequence of CASP9, CASP3, or CASP8 activation. Further experiments are required to increase our understanding of the relationship between intracellular cell-death signaling and organismal homeostasis.

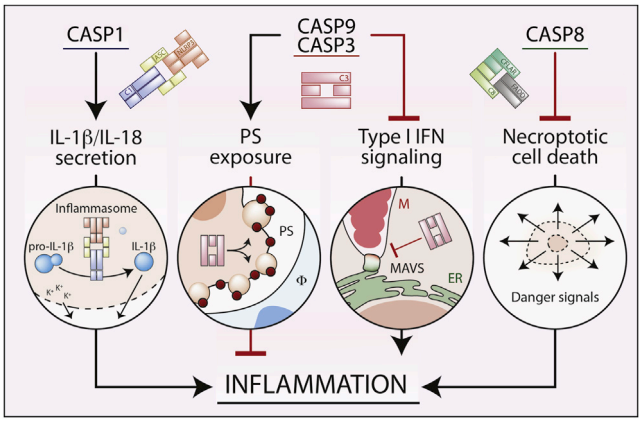

Figure 1. Pro- and Anti-inflammatory Functions of Caspases

In the course of necrotic cell death, activation of the NLRP3 inflammasome (which contains CASP1) exacerbates inflammation through proteolytic conversion of pro-IL-1β into biologically active IL-1β. Conversely, CASP8 tonically suppresses necroptosis, which is associated with the release of several danger signals, limiting the inflammatory potential of cell death. Similarly, upon activation by CASP9, CASP3 exerts anti-inflammatory functions by promoting the exposure of phosphatidylserine (PS) on the surface of dying cells and by inhibiting type I interferon (IFN) responses driven by MAVS. Thus, caspases have a prominent impact on how the organism perceives regulated cell death. Abbreviations are as follows: CFLAR, CASP8 and FADD-like apoptosis regulator; ER, endoplasmic reticulum; FADD, Fas (TNFRSF6)-associated via death domain; M, mitochondria; Φ, macrophage.

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